

What is claimed is:

1. A nucleotide sequence comprising a maize optimized coding sequence which encodes a protein capable of killing an insect.
2. A nucleotide sequence of claim 1, wherein said coding sequence encodes a B.t. protein.
3. A nucleotide sequence of claim 2, wherein said coding sequence encodes a CryIA(b) protein.
4. A nucleotide sequence of claim 3, wherein said coding sequence comprises Sequence 3, Sequence 4, or the sequence set forth in Fig. 7.
5. A nucleotide sequence of claim 3, wherein coding sequence encodes a CryIA(b) protein which is heat stable compared to a native CryIA(b) protein.
6. A nucleotide sequence of claim 5, wherein said coding sequence comprises a sequence selected from the group of sequences consisting of Fig. 9, Fig. 11, Fig. 13 and Fig. 15.
7. A nucleotide sequence of claim 2, wherein said coding sequence encodes a CryIB or a CryIA(c) protein.
8. A nucleotide sequence of claim 7, wherein said coding sequence comprises the sequence encoding a CryIB protein set forth in Fig. 6.
9. A nucleotide sequence of claim 1, further comprising a first promoter capable of directing expression of a nucleotide sequence in a plant cell, operably linked to said coding sequence.
10. A nucleotide sequence of claim 9, wherein said promoter is capable of directing expression of the associated coding sequence in a maize cell.
11. A nucleotide sequence of claim 9, wherein said promoter is selected from the group consisting of inducible promoters, constitutive promoters, temporal or developmentally-regulated promoters, tissue-preferred, and tissue-specific promoters.

12. A nucleotide sequence of claim 9, wherein said promoter is selected from the group consisting of a CaMV 35S promoter, CaMV 19S promoter, a PEP carboxylase promoter, a pith-preferred promoter, and a pollen-specific promoter.

13. A nucleotide sequence of claim 9, wherein said promoter is a pith-preferred promoter comprising the DNA sequence set forth in Fig. 24.

14. A nucleotide sequence of claim 9, wherein said promoter is a pollen-specific promoter comprising the DNA sequence set forth in Fig. 35.

15. A nucleotide sequence of claim 9, further comprising a second promoter capable of directing expression of an associated coding sequence in a plant cell, operatively linked to a second coding sequence.

17. A nucleotide sequence of claim 15, wherein said second promoter is selected from the group consisting of inducible promoters, constitutive promoters, temporal or developmentally-regulated promoters, tissue-preferred, and tissue-specific promoters.

18. A nucleotide sequence of claim 15, wherein said second promoter is selected from the group consisting of a CaMV 35S promoter, CaMV 19S promoter, a PEP carboxylase promoter, a pith-preferred promoter, and a pollen-specific promoter.

21. A nucleotide sequence of claim 15, wherein said second coding sequence is a plant optimized coding sequence which encodes a protein capable of killing an insect.

22. A nucleotide sequence of claim 21, wherein said second coding sequence encodes a B.t. protein.

23. A nucleotide sequence of claim 22, wherein said second coding sequence encodes a CryIA(b) protein.

29. A nucleotide sequence of claim 15, wherein said second coding sequence is a marker gene.

30. A recombinant vector, comprising a nucleotide sequence of claim 9.

31. A recombinant vector, comprising a nucleotide sequence of claim 9, wherein said coding sequence encodes a B.t. protein.

32. A recombinant vector of claim 31, wherein the B.t. protein is a CryIA(b) protein.

33. A recombinant vector, comprising a nucleotide sequence of claim 15.

34. A recombinant vector, comprising a nucleotide sequence of claim 18.

35. A recombinant vector, comprising a nucleotide sequence of claim 22.

36. A plant stably transformed with a nucleotide sequence of claim 9.

37. A plant stably transformed with a nucleotide sequence of claim 12.

38. A plant of claim 36, wherein said coding sequence encodes a B.t. protein.

39. A plant of claim 36, wherein said protein is expressed in said plant in an amount sufficient to control Lepidopteran or Coleopteran insects.

40. A plant of claim 38, wherein the B.t. protein is a CryIA(b) protein.

41. A plant of claim 38, which expresses the B.t. insecticidal protein in an amount sufficient to control Lepidopteran or Coleopteran pests.

42. A plant of claim 38, wherein the amount is sufficient to control insects selected from the group consisting of European corn borer, Sugarcane borer, stalk borers, cutworms, armyworms, rootworms, wireworms and aphids.

43. A plant stably transformed with a nucleotide sequence of claim 15.

44. A plant stably transformed with a nucleotide sequence of claim 18.

45. A plant of claim 43, wherein the first and second coding sequences each encode a B.t. protein.

46. A plant of claim 38, which expresses the B.t. insecticidal proteins in an amount sufficient to control Lepidopteran or Coleopteran pests.

47. A plant of claim 46, wherein the amount is sufficient to control stalk borers.

48. An isolated and purified promoter capable of directing pith-preferred expression of an associated structural gene in a plant.

49. A promoter of claim 48, isolated from a monocot.

50. A promoter of claim 48, isolated from a maize plant.

51. A promoter of claim 48, isolated from a plant tryptophan synthase-alpha (TrpA) subunit gene.

52. A promoter of claim 51, isolated from a maize tryptophan synthase-alpha (TrpA) subunit gene.

53. A promoter of claim 48, comprising the sequence set forth in Figure 24.

54. A recombinant DNA molecule comprising a promoter of claim 48, operably associated with a structural gene encoding a protein of interest.

55. A recombinant DNA molecule of claim 54, wherein said structural gene encodes an insecticidal protein.

56. A recombinant DNA molecule of claim 55, wherein said structural gene encodes a *Bacillus thuringiensis* protein.

57. A vector, comprising a recombinant DNA molecule of claim 54.

58. A vector of claim 57, wherein said structural gene encodes an insecticidal protein.

59. A vector of claim 57, wherein said structural gene encodes a *Bacillus thuringiensis* protein.

60. A vector, comprising at least two recombinant DNA molecules of claim 54, wherein at least one of the two structural genes encodes an insecticidal protein.

61. A plant stably transformed with recombinant DNA molecule of claim 54.

62. A plant of claim 61, which is a maize plant.

63. A purified promoter capable of directing pollen-specific expression of an associated structural gene in a plant, wherein said promoter is isolated from a plant calcium-dependent phosphate kinase (CDPK) gene.

64. A promoter of claim 63, isolated from a monocot CDPK gene.

65. A promoter of claim 63, isolated from a maize CDPK gene.

66. A promoter of claim 65, comprising the sequence set forth in Figure 35.

67. A recombinant DNA molecule, comprising a promoter of claim 63, operably associated with a structural gene encoding a protein of interest.

68. A recombinant DNA molecule, of claim 67, wherein said structural gene encodes an insecticidal protein.

69. A recombinant DNA molecule of claim 68, wherein said structural gene encodes a *Bacillus thuringiensis* protein.

70. A vector, comprising at least one recombinant DNA molecule of claim 67.

71. A vector of claim 70, wherein said structural gene encodes an insecticidal protein.

72. A vector of claim 71, wherein said structural gene encodes a *Bacillus thuringiensis* protein.

73. A vector of claim 70, comprising two recombinant DNA molecules, wherein at least one of the two structural genes encodes an insecticidal protein.

74. A plant stably transformed with a recombinant DNA molecule of claim 67.
75. A plant of claim 74, which is maize plant.
76. A maize plant stably transformed with at least one recombinant DNA molecule;  
wherein said DNA molecule comprises a promoter operably linked to a nucleotide  
sequence encoding an insecticidal protein.  
wherein said promoter is capable of directing tissue-preferred or tissue-specific  
expression of said gene in said maize plant.
77. A maize plant of claim 76, wherein said gene encodes a *Bacillus thuringiensis*  
protein.
78. A plant of claim 76, wherein said promoter is obtained from a monocot.
79. A plant of claim 78, wherein said monocot is maize.
80. A maize plant of claim 76, wherein said promoter is capable of directing  
pith-preferred expression of said gene in said maize plant.
81. A plant of claim 76, wherein said promoter is obtained from a plant tryptophan  
synthase-alpha subunit gene.
82. A plant of claim 76, wherein said promoter is obtained from a maize tryptophan  
synthase-alpha subunit gene.
83. A plant of claim 82, wherein said promoter comprises the sequence set forth in  
Figure 24.
84. A plant of claim 76, wherein said promoter is capable of directing green  
tissue-specific expression of said gene in said maize plant.
85. A plant of claim 84, wherein said promoter is a PEP carboxylase promoter.
86. A maize plant of claim 76, wherein said promoter is capable of directing  
pollen-specific expression of said gene in said maize plant.

87. A maize plant of claim 76, wherein said promoter is obtained from a plant calcium-dependent phosphate kinase gene.

88. A maize plant of claim 76, wherein said promoter is obtained from a maize calcium-dependent phosphate kinase gene.

89. A maize plant of claim 76, wherein said promoter comprises the sequence set forth in Figure 35.

90. A method of producing a maize optimized coding sequence for an insecticidal B.t. protein, comprising:

determining the amino acid sequence of a predetermined insecticidal B.t. protein, and  
altering the coding sequence of the protein by substituting codons which are most preferred in maize for corresponding native codons.

91. A method of protecting a maize plant against at least one insect pest, comprising:  
stably transforming a maize plant with at least one nucleotide sequence of claim 9, wherein the coding sequence encodes an insecticidal protein; whereby the transformed maize plant expresses the insecticidal protein in an amount sufficient to protect the plant against the pest.

92. The method of claim 91, wherein said insecticidal protein is a B.t. protein.

93. The method of claim 91, wherein said promoter is a tissue-specific or a tissue-preferred promoter.

94. A plant which has been stably transformed with a nucleotide sequence wherein said nucleotide sequence comprises a maize optimized coding sequence for an insecticidal protein, wherein said protein is expressed in said transformed plant at least 100 fold greater than expression of the protein using a native coding sequence.

95. A transgenic maize seed comprising a chimeric gene comprising a heterologous promoter sequence operatively linked to a synthetic DNA coding sequence that encodes a *Bacillus thuringiensis* (Bt) insecticidal protein selected for optimized expression in a plant, wherein said synthetic DNA coding sequence is produced by a method comprising:

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- (a) obtaining the amino acid sequence of said Bt insecticidal protein;
  - (b) reverse-translating said amino acid sequence into a synthetic DNA coding sequence comprising a sufficient number of the single codons that most frequently encode each amino acid in maize, wherein said synthetic DNA coding sequence has at least about 60% G+C content, and wherein the single codons that most frequently encode each amino acid in maize are determinable by
    - (i) pooling a plurality of gene sequences from maize,
    - (ii) calculating a codon usage profile from said pooled maize gene sequences, and
    - (iii) determining which single codon most frequently encodes each amino acid in maize; and
  - (c) synthesizing said DNA coding sequence.

96. A transgenic maize seed according to claim 95, wherein said *Bacillus thuringiensis* insecticidal protein is CryIA(b).

97. A transgenic maize seed according to claim 95, wherein said *Bacillus thuringiensis* insecticidal protein is CryIB.

98. A transgenic maize plant grown from the transgenic maize seed of claim 95.

99. A method of controlling insect pests, comprising contacting the insect pests with the transgenic plant according to claim 98.

100. The method of claim 99, wherein the insect pests are lepidopteran insect pests.

101. The method of claim 100, wherein the insect pests are European corn borers.

102. A method of producing an insect-resistant maize plant, comprising growing the transgenic maize seed of claim 95, wherein said synthetic DNA coding sequence is expressed in said plant in an effective amount to control insect pests.

103. The method of claim 102, wherein the insect pests are lepidopteran insect pests.

104. The method of claim 103, wherein the insect pests are European corn borers.



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105. A transgenic plant seed comprising a chimeric gene comprising a heterologous promoter sequence operatively linked to a synthetic DNA coding sequence that encodes a *Bacillus thuringiensis* (Bt) insecticidal protein selected for optimized expression in a plant, wherein said synthetic DNA coding sequence is produced by a method comprising:

- (a) obtaining the amino acid sequence of said Bt insecticidal protein;
- (b) reverse-translating said amino acid sequence into a synthetic DNA coding sequence comprising a sufficient number of the following codons: Ala, GCC; Arg, CGC; Asn, AAC; Asp, GAC; Cys, TGC; Gln, CAG; Glu, GAG; Gly, GGC; His, CAC; Ile, ATC; Leu, CTG; Lys, AAG; Met, ATG; Phe, TTC; Pro, CCC; Ser, AGC; Thr, ACC; Trp, TGG; Tyr, TAC; and Val, GTG; such that said synthetic DNA coding sequence has at least about 60% G+C content; and
- (c) synthesizing said DNA coding sequence.

106. A transgenic plant seed according to claim 105, wherein said *Bacillus thuringiensis* insecticidal protein is CryIA(b).

107. A transgenic plant seed according to claim 105, wherein said *Bacillus thuringiensis* insecticidal protein is CryIB.

108. A transgenic plant grown from the transgenic plant seed of claim 105.

109. A transgenic plant according to claim 108, which is maize.

110. A method of controlling insect pests, comprising contacting the insect pests with the transgenic plant according to claim 108.

111. The method of claim 110, wherein the insect pests are lepidopteran insect pests.

112. The method of claim 110, wherein said transgenic plant is maize.

113. The method of claim 112, wherein the insect pests are European corn borers.

114. A method of producing an insect-resistant plant, comprising growing the transgenic seed of claim 105, wherein said synthetic DNA coding sequence is expressible in said plant in an effective amount to control insect pests.

115. The method of claim 114, wherein the insect pests are lepidopteran insect pests.

116. The method of claim 114, wherein said plant is maize.

117. The method of claim 116, wherein the insect pests are European corn borers.

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